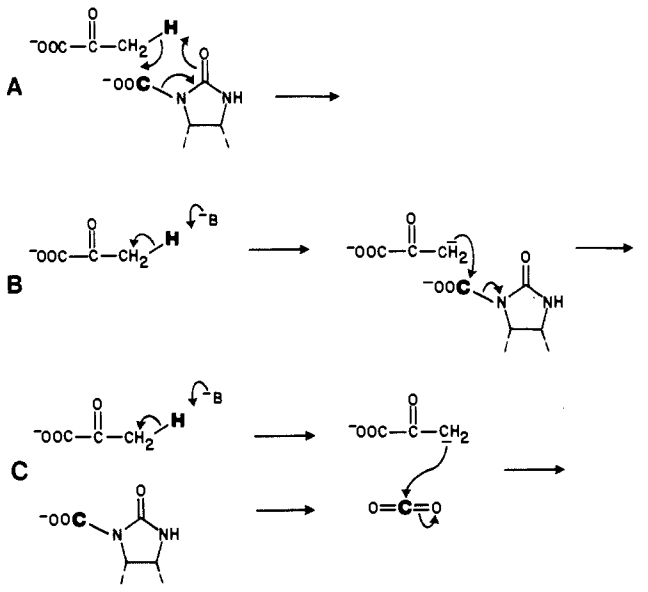

Figure 1. Pathways for biotin-dependent carboxylations. The carbon and the hydrogen directly involved in the isotope effects are shown in heavy type.

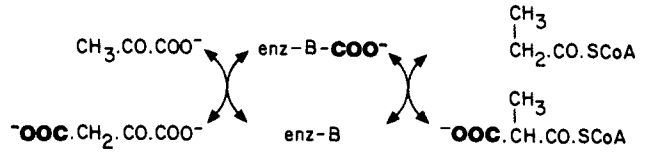

Figure 2. Reaction catalyzed by transcarboxylase. The enzyme is enz, and biotin is B.

isotope effect is observed, therefore, the reaction must be stepwise, but if both are seen, the reaction could be either concerted or stepwise. In 1975, Cheung et al.[12] showed that when pyruvate-d_3 is the substrate for transcarboxylase (Figure 2), a ^2H kinetic isotope effect is observed. We have now measured the ^{13}C isotope effect in the pyruvate carboxylation half-reaction[13] catalyzed by transcarboxylase to be 1.0227 ± 0.0007.[14] These results alone do not allow us to distinguish between concerted and stepwise paths. The distinction is possible, however, from a determination of the ^{13}C isotope effect using deuterated pyruvate. If the reaction is stepwise, deuteration of the pyruvate will slow the deprotonation step, making the subsequent carboxylation step less rate-limiting and thus lowering the observed ^{13}C isotope effect.[9,10,15] If the reaction is concerted, the overall reaction rate will be slowed by deuteration, but the ^{13}C isotope effect will remain unchanged.[9,10,15]

When pyruvate-d_3[16] is the substrate in the transcarboxylase reaction, the ^{13}C isotope effect is 1.0141 ± 0.001. That is, deuterium substitution in the pyruvate *reduces* the ^{13}C isotope effect from 2.3% to 1.4%, which means that *proton removal and carboxy group addition occur in different steps in the transcarboxylase-catalyzed reaction.* The effect of pyruvate deuteration on the ^{13}C kinetic isotope effect can be predicted[15] from the size of the observed ^{13}C isotope effect for protiopyruvate (1.0227), the measured deuterium isotope effect (1.4), and the ground-state fractionation factors for pyruvate[17] and the enzyme base that abstracts the proton.[15] The predicted value is 1.0136, which is gratifyingly close to our observed value of 1.0141. While the present experiments do not distinguish between the two stepwise paths (Figure 1B,C), the concerted pathway (Figure 1A) can, at last, be eliminated from further consideration.[18]

Acknowledgment. We are grateful to Joel Belasco for germinal contributions to the double isotope fractionation test, to Jeff Hermes and Peter Leadlay for helpful discussions, and to the National Science Foundation for support.

(12) Cheung, Y. F.; Fung, C. H.; Walsh, C. T. *Biochemistry* **1975**, *14*, 2981-2986. These workers obtained a deuterium isotope effect of 2.1 in V_{max} and in V_{max}/K_m. We have redetermined this quantity using pyruvate-d_3 under conditions similar to those used for the ^{13}C isotope effect measurement[13,14] and find a value of 1.4 ± 0.1. This discrepancy presumably derives from differences in pH, buffer, solvent composition, and cosubstrates.[14]

(13) To ensure that the measured ^{13}C isotope effect only relates to the half-reaction involving pyruvate carboxylation, the other half-reaction was maintained at equilibrium, and pyruvate was added at a rate such that pyruvate carboxylation was always at least 10 times slower than the carboxylation of acetyl-CoA.[14]

(14) Malonyl-CoA and acetyl-CoA were used for the second half-reaction instead of *S*-methylmalonyl-CoA and propionyl-CoA. The former substrates react about 0.7 times as fast as the latter pair (see also: Wood, H. G.; Jacobson, G., Gerwin, B. I.; Northrop, D. B. *Methods Enzymol.* **1969**, *13*, 215-230). The exchange rate between [^{14}C]acetyl-CoA and malonyl-CoA under the conditions used was at least 10 times faster than the rate of oxalacetate formation. Malonyl-CoA (250 μmol) and acetyl-CoA (250 μmol) were incubated with transcarboxylase[11] (20 units) at 30 °C for 30-45 min in 0.35 M potassium phosphate buffer, pH 6.8 (41.5 mL). The reaction was initiated by addition of pyruvate (6 μmol) and 1 μmol was added each minute for 44 min. The product oxalacetate was concomitantly converted to malate with NADH (125 μmol) and malate dehydrogenase (250 units). The reaction was followed by monitoring the change in absorbance at 340 nm. At the end of the reaction, malate was isolated by the procedure of O'Leary et al. (O'Leary, M.H.; Rife, J.E.; Slater, J.D. *Biochemistry* **1981**, *20*, 7308-7314), after removal of inorganic phosphate by precipitation as Li_3PO_4 and removal of excess Li^+ on an Amberlite IR-120(H^+) column. The purified malate was decarboxylated according to the procedure of: Hermes, J. D.; Roeske, C. A.; O'Leary, M. H.; Cleland, W. W. *Biochemistry* **1982**, *21*, 5106-5114. The CO_2 was isolated (O'Leary, M. H. *Methods Enzymol.* **1980**, *64*, 83-104). The ^{13}C:^{12}C ratio (corrected for ^{17}O content) was determined by isotope ratio mass spectrometry (Kruger Enterprises, Cambridge, MA).

(15) On the reasonable basis that each isotope fractionates independently of the other, we know that $[^{13}(V/K)_H - 1]/[^{13}(V/K)_D - 1] = {}^D(V/K)/{}^DK_{eq}$.[10] From the measured values of $^{13}(V/K)_H$ of 1.0227 and of $^D(V/K)$ of 1.4 and the knowledge of $^DK_{eq}$ (the deuterium starts in pyruvate with a fractionation factor of 0.84,[17] and ends up on an enzymic base with a presumed fractionation factor of 1.0), we can calculate the value of $^{13}(V/K)_D$ to be 1.0136.

(16) Cook, P. F.; Blanchard, J. S.; Cleland, W. W. *Biochemistry* **1980**, *19*, 4853-4858.

(17) Cleland, W. W. *Methods Enzymol.* **1980**, *64*, 104-125.

(18) Attwood and Cleland (Attwood, P. V.; Cleland, W. W., private communication) have recently arrived at the same conclusion for pyruvate carboxylase, by measuring the ^{13}C isotope effects for the enzymic decarboxylation of oxalacetate in solution in H_2O and in D_2O.

η^5 to η^3 Conversion in Indenyliridium Complexes

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Recently, several reports have suggested that ring slippage of cyclopentadienyl (Cp) metal complexes in which the Cp ring changes its bonding mode from η^5 to η^3 may be the mechanism by which a coordination site is opened in certain reactions.[1] Indenyl metal (IndM) complexes are more reactive for ligand substitution than their cyclopentadienyl analogues, presumably because the η^3 mode of bonding is stabilized by aromatization of the benzene ring.[2] This enhanced reactivity has been termed the "indenyl effect".[2] Even though there is a structurally characterized

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(1) (a) Rerek, M. E.; Basolo, F. *Organometallics* **1983**, *2*, 372. (b) Yang, G. K.; Bergman, R. G. *Organometallics* **1985**, *4*, 129. (c) Rest, A. J.; Whitwell, I.; Graham, W. A. G.; Hoyano, J. K.; McMaster, A. D. *J. Chem. Soc., Chem. Commun.* **1984**, 624.

(2) (a) Hart-Davis, A. J.; Mawby, R. J. *J. Chem. Soc. A* **1969** 2403. (b) Hart-Davis, A. J.; White, C.; Mawby, R. J. *Inorg. Chim. Acta* **1970**, *4*, 441. (c) White, C.; Mawby, R. J. *Inorg. Chim. Acta* **1970**, *4*, 261. (d) Jones, D. J.; Mawby, R. J. *Inorg. Chim. Acta* **1972**, *6*, 157. (e) Eshtiagh-Hosseini, H.; Nixon, J. F. *J. Less-Common Met.* **1978**, *61*, 107. (f) Rerek, M. E.; Ji, L.-N.; Basolo, F. *J. Chem. Soc., Chem. Commun.* **1983**, 1208. (g) Ji, L.-N.; Rerek, M. E.; Basolo, F. *Organometallics* **1984**, *3*, 740.

0002-7863/86/1508-0329$01.50/0

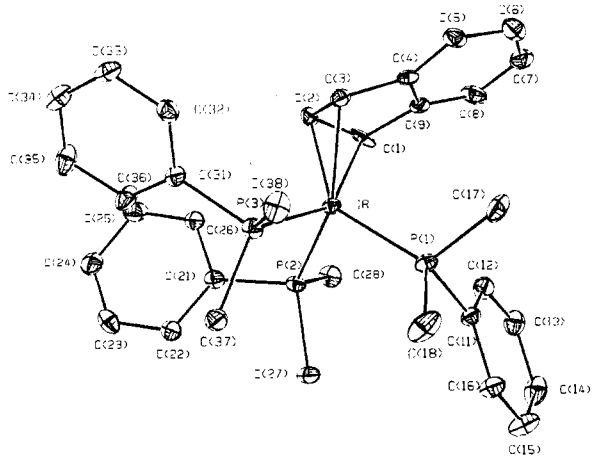

Figure 1. ORTEP Plot of **3b**. Hydrogen atoms omitted for clarity. Some important bond distances: Ir–C1, 2.235 (14); Ir–C2, 2.047 (8); Ir–C3, 2.236 (13); Ir–C4, 3.036 (8); Ir–C9, 3.014 (8); C1–C2, 1.455 (14); C2–C3, 1.439 (13); C3–C4, 1.472(12); C4–C9, 1.408(11); C9–C1, 1.460 (12); Ir–P1, 2.316 (2); Ir–P2, 2.288 (2); Ir–P3, 2.266 (2).

(η^3-indenyl)metal complex of tungsten,[3] this material was not synthesized via nucleophile-induced ring slippage of an (η^5-indenyl)metal precursor. In a very recent paper, Casey and O'Connor report on their attempts to isolate or observe η^3-indenyl intermediates in the reaction chemistry of (η^5-indenyl)tricarbonylrhenium, and they noted slippage of the η^5-indenyl ring to η^1 coordination without observing the presumed η^3 intermediate.[4] In this paper, we wish to report our results on some aspects of indenyliridium chemistry, including isolation of an η^3-indenyl complex via an η^5 to η^3 shift.

Pale yellow (η^5-indenyl)bis(cyclooctene)iridium (IndIr(COE)$_2$, (**1**) may be prepared in good yield from lithium indenide and bis(cyclooctene)chloroiridium dimer (eq 1).[5]

$$0.5\{\mathrm{Ir(COE)_2Cl}\}_2 + \mathrm{Li^+Ind^-} \rightarrow \underset{\mathbf{1}}{\eta^5\text{-IndIr(COE)}_2} + \mathrm{LiCl} \qquad (1)$$

Reaction of **1** with L = CO and triethylphosphine (PEt_3) results in the clean displacement of the cyclooctene and the formation of (η^5-indenyl)bis(ligand)iridium complexes **2a,b** (eq 2).[6]

$$\underset{\mathbf{1}}{\eta^5\text{-IndIr(COE)}_2} + \text{excess L} \rightarrow \underset{\begin{array}{l}\mathbf{2a},\ \mathrm{L = CO}\\ \mathbf{2b},\ \mathrm{L = PEt_3}\end{array}}{\eta^5\text{-IndIrL}_2} \qquad (2)$$

However, when small phosphine ligands such as trimethylphosphine (PMe_3) (cone angle 118°)[8] and dimethylphenylphosphine ($PPhMe_2$) (cone angle 122°)[8] are allowed to react with **1**, new red, crystalline complexes, **3a,b**, are isolated having the form (η^3-indenyl)tris(ligand)iridium (eq 3).

$$\underset{\mathbf{1}}{\eta^5\text{-IndIr(COE)}_2} + 3\mathrm{L} \rightarrow \underset{\begin{array}{l}\mathbf{3a},\ \mathrm{L = PMe_3}\\ \mathbf{3b},\ \mathrm{L = PPhMe_2}\end{array}}{\eta^3\text{-IndIrL}_3} \qquad (3)$$

Support for the formulations of **3a,b** comes from elemental analysis as well as from ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy.[9] In the case of **3b**, crystals suitable for a single-crystal X-ray diffraction study could be grown and the results confirm the η^3 nature of the indenyl ligand.[10] An ORTEP plot of **3b** is shown in Figure 1 along with some selected bond distances and angles. The Ir–C(4) and Ir–C(9) bond distances of 3.036 (8) and 3.014 (8) Å, respectively, are clearly out of the bonding range, and the 28° angle between the plane defined by C(1), C(2), and C(3) and that defined by the indenyl 6-membered ring is also worthy of note in that context. Overall, the structure of the η^3-indenyl ligand in this complex is quite similar to the previously structurally characterized η^3-indenyl complex of tungsten.[3]

The changes in the NMR spectra of the indenyl ligand on going from η^5 to η^3 coordination are quite striking. For example, in the ^{1}H NMR spectrum of (η^5-indenyl)bis(cyclooctene)iridium, H1 and H3 display a doublet at δ 4.89 while H2 gives rise to a triplet at δ 5.82. On going to η^3 in the case of (η^3-indenyl)tris(trimethylphosphine)iridium, the resonance for H1 and H3 undergoes a large upfield shift to δ 2.77, while the resonance for H2 undergoes a downfield shift to δ 7.09. While the upfield shift for H1 and H3 is consistent both with the formulation of an η^3-indenyl as an allyl ligand as well as with the placement of more electron density on the iridium with the PMe_3 ligands, the downfield shift of H2 is surprising.[11] Both the chemical shift of H2 and the nonplanarity of the η^3-indenyl ligand suggest that the π-allyl description for this form of the ligand is an oversimplification of the actual bonding. The chemical shift of carbons C4 and C9 in the ^{13}C NMR spectrum is also unusual, being a good indication of η^3 bonding for the indenyl ligand. The chemical shift for C4,C9 in the η^5, bis(phosphine) complex **2b** is δ 121.15, while that for C4,C9 in the η^3 complex **3a** is δ 156.7, in a downfield region indicating no interaction with a metal.[12]

To our knowledge, this is the first example of the interception of a complex containing an η^3-indenyl ligand by the reaction between a complex with an η^5-indenyl ring and incoming ligands.

(3) Nesmeyanov, A. N.; Ustynyuk, N. A.; Makarova, L. G.; Andrianov, V. G.; Struchkov, Yu. T.; Andrae, S.; Ustynyuk, Yu. A.; Malyugina, S. C. *J. Organomet. Chem.* **1978**, *159*, 189.

(4) Casey, C. P.; O'Connor, J. M. *Organometallics* **1985**, *4*, 384.

(5) IndIr(COE)$_2$: (the NMR assignments follow the numbering scheme used in the ORTEP plot) ^{1}H NMR (400 MHz, C_6D_6) δ 1.29–1.63 (complex m, 24 H, cyclooctene CH_2), 2.04–2.07 (m, 4 H, cyclooctene CH), 4.88 (d, J = 2.2 Hz, 2 H, indenyl H1/H3), 5.82 (t, J = 2.2 Hz, 1 H, indenyl H2), 7.10–7.16 (m, 4H, indenyl H5–H8); ^{13}C{^{1}H} NMR (22 MHz, C_6D_6) δ 26.96, 32.98 (cyclooctene CH_2, with two methylenes apparently overlapped) 51.62 (cyclooctene CH), 78.27 (indenyl C1–C3), 86.46 (indenyl C2), 111.01 (indenyl C4–C9), 120.51, 123.73 (indenyl C5–C8). Anal. ($C_{25}H_{35}Ir$) C, H, Ir.

(6) (a) IndIr(CO)$_2$: ^{1}H NMR (60 Mhz, C_6D_6) δ 5.1 (d, J = 2.2 Hz, 2 H, indenyl H1–H3), 5.5 (t, J = 2.2 Hz, 1 H indenyl H2), 6.8 (s, 4 H, indenyl H5–H8); IR spectrum (pentane) ν_{CO} 2042 (s), and 1979 (s) cm^{-1}. Anal. ($C_{11}H_7IrO_2$) C, H, Ir. (b) IndIr(PEt$_3$)$_2$:[7] ^{1}H NMR (400 MHz, C_6D_6) δ 0.77 (dt, J_{HH} = 7.34, J_{PH} = 15.4 Hz, 18 H, PCH_2CH_3), 1.41 (dq, J_{HH} = 7.34, J_{PH} = 7.3 Hz, 12 H, PCH_2CH_3), 4.91 (d, J = 2.2 Hz, 2 H, indenyl H1–H3), 6.31 (m, J_{HH} = 2.2, J_{PH} = 2.9 Hz, 1 H, indenyl H2), 7.06 (m, 4 H, indenyl H5–H8); ^{13}C{^{1}H} NMR (C_6D_6, 22.5 MHz) δ 8.73 (s, PCH_2CH_3), 23.73 (m, PCH_2CH_3), 64.73 (m, indenyl C1–C3 with apparent coupling to two phosphines), 89.60 (s, C2), 113.96 (s, C6–C7), 120.11(s, C5–C8), 121.15 (s, C4–C9); ^{31}P{^{1}H} NMR (C_6D_6) δ −4.61. Anal. ($C_{21}H_{37}IrP_2$) C, H, Ir, P.

(7) The rotation about the indenyl–iridium bond appears to have a relatively high barrier. In both the η^5- and η^3-indenyl complexes with phosphine ligands, the room temperature spectra reported in this paper show intermediate rates of rotation about the indenyl–iridium bond. As such, the multiplicities of the indenyl protons H1, H2, and H3, and the carbons C1, C2, and C3, do not reflect limiting spectra and true P–H or P–C coupling constants. The multiplicities are reported here without reference to coupling constants. These values from the low-temperature limiting spectra, along with the temperature dependence of these spectra, will be reported completely in the full paper.

(8) Tolman, C. A. *Chem. Rev.* **1977**, *77*, 313.

(9) (a) IndIr(PMe$_3$)$_3$:[7] ^{1}H NMR (400 MHz, C_6D_6) δ 1.07 (br, s, 27 H, PCH_3), 2.78 (d, J = 3.1 Hz, 2 H, indenyl H1–H3), 6.55 (m, 4 H, indenyl H5–H8), 7.09 (dt, J_{HH} = 3.1 Hz and apparent coupling to only one phosphorus, 1 H, indenyl H2); ^{13}C{^{1}H} NMR (22 Mhz, C_6D_6) δ 22.6 (d, J_{PC} = 25.3 Hz, PCH_3), 37.8 (br d, apparent coupling to only one phosphorus, indenyl C1–C3), 69.4 (s, indenyl C2), 115.2, 119.4 (s, indenyl C5–C8), 156.7 (s, indenyl C4–C9); ^{31}P{^{1}H} NMR (36.2 MHz, toluene-d_8, 30 °C) δ −44.25 (br, P1), −50.58 (br, P2 and P3). Anal. ($C_{18}H_{34}IrP_3$) C, H, Ir, P. (b) IndIr(PPhMe$_2$)^7 ^{1}H NMR (400 MHz, C_6D_6) δ 1.21 (br s, 18 H, PCH_3), 3.08 (d, J = 2.2 Hz, 2 H, indenyl H1–H3), 6.64 (m, 4 H, indenyl H5–H8), 7.01–7.60 (m, 16 H, phenyl and indenyl H2); ^{13}C{^{1}H} NMR (C_6D_6, 22.5 MHz) δ 20.22 (d, J_{PC} = 25.3 Hz, PCH_3), 39.68 (m, indenyl C1–C3), 75.47 (s, indenyl C2), 116.04 and 120.20 (indenyl C5–C8), 128.00, 129.99, 130.43, 131.38 (PC_6H_5), 156.43 (C4/C9); ^{31}P{^{1}H} NMR (C_6D_6, 36.2 MHz, 30 °C) δ −35.23 (v br, P1–P3). Anal. ($C_{33}H_{40}IrP_3$) C, H, Ir, P.

(10) Crystal data: IndIr(PMe$_2$Ph)$_3$, **3b**, $C2/c$, a = 30.943 (13) Å b = 11.873 (2) Å, c = 17.144 (4) Å, β = 104.77 (3)°, V = 6090.3 (7) Å^3, Z = 8, ρ(calcd) = 1.574 g/cm^3, R = 0.047, R = 0.057, all non-hydrogen atoms anisotropic.

(11) One referee suggested that these spectral changes may reflect the addition of phosphine ligands and not necessarily the change in hapticity. We have found that indenylbis(phosphine)iridium complexes are much more severely distorted toward the η^3 mode of bonding, so that it is difficult to separate these two factors. We will discuss these results in a future report. Merola, J. S., unpublished results.

(12) Kohler, F. H. *Chem. Ber.* **1977**, *107*, 570. (b) Workers at Du Pont have elaborated on the study of the ^{13}C chemical shift of the indenyl C4,C9 carbons as a diagnostic of the hapticity changes of the indenyl ligand and have confirmed that this is a parameter quite sensitive to η^5-to-η^3 distortions of the indenyl ligand. Baker, T., personal communication.

We are continuing our studies of these η^3-indenyl complexes in order to more completely understand the solution dynamics and the bonding in these compounds.

Acknowledgment. We acknowledge the contributions of Dr. R. V. Kastrup for many of the NMR spectra for this paper. We thank Drs. T. Baker and T. Marder for communication of their results prior to publication. We also thank Exxon Research an Engineering Co. for support of this work and for permission to publish it.

Supplementary Material Available: Complete details of the X-ray diffraction experiment, listings of positional and thermal parameters, bond angles and distances, and observed and calculated structure factors for **3b** (39 pages). Ordering information is given on any current masthead page.

Biosynthesis of the Antibiotic Reductiomycin

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The antibiotic reductiomycin (**1**), a metabolite of *Streptomyces xanthochromogenus* (AM 6201),[1] consists of two unique structural units. One is a 2-amino-3-hydroxycyclopent-2-enone moiety, which is also found in the antibiotics asukamycin,[2] manumycin,[3] moenomycin,[4] senecarcin A,[5] virustomycin A,[6] bafilomycin B_1,[7] and antibiotic L-155,175.[8] The other is an unusual acetoxydihydrofuran carrying an acrylic acid side chain. In this paper we report results which establish the biosynthetic origin of these two moieties.

Labeled precursors were fed to 24-h old cultures of *S. xanthochromogenus* AM 6201 grown in 100 mL of medium (2% glucose, 2% soybean meal, 0.3% NaCl, pH 7.0) in 500-mL baffled Erlenmeyer flasks (29 °C, 300 rpm rotary shaking). Cultures were harvested 48 h later and **1** (about 150 mg/L) was extracted (ethyl acetate) from the acidified broth, purified by preparative layer chromatography (silica gel GF, $CHCl_3$–acetone 9:2), quantitated by HPLC (C-18, 5 μm, CH_3OH–H_2O 4:1), and subjected to liquid scintillation counting (Beckman LS 7500) and/or ^{13}C NMR spectroscopy (Bruker WM 300, 7.1 T, solvent $CDCl_3$). The ^{13}C NMR assignments of **1** are based on multiplicity, chemical shift theory, and characteristics of the line shapes and intensities in different solvents.

We[9] recently reported results which suggested formation of the 2-amino-3-hydroxycyclopent-2-enone moiety of asukamycin by an intramolecular cyclization of 5-aminolevulinic acid (**2**). Feeding experiments with [^{14}C]glycine point to a similar origin of this moiety in **1**. Thus, [2-^{14}C]glycine, but not [1-^{14}C]glycine, is efficiently incorporated into **1** (specific incorporation 20.9% vs. 0.8%). As in the case of asukamycin,[9] [1(4)-^{14}C]succinic acid and [5-^{14}C]-**2** were incorporated significantly (2.3% and 1.8%) but less efficiently than [2-^{14}C]glycine. The origin of the 2-amino-3-hydroxycyclopent-2-enone moiety from **2** was unequivocally proven by feeding [4,5-$^{13}C_2$]-**2** (100 mg, 90% ^{13}C per labeled carbon, Cambridge Isotopes, Woburn, MA) and observing the expected enrichments and couplings in the ^{13}C NMR spectrum of the resulting **1** (55 mg) (Table I). C-2 is twice as enriched as C-1 and C-3; in half the labeled molecules it is coupled to C-1 and in the other half to C-3. As suggested previously,[9] it seems likely that the cyclization of **2** involves an acylation by C-5 of a pyridoxal phosphate-generated α-carbanion.

Inspection of the dihydrofuran moiety suggests the possibility that the entire nine-carbon assembly, including the acetoxy group, could be derived by a ring cleavage of phenylalanine or tyrosine or a derivative thereof. In agreement with such an assumption, both ^{14}C-labeled shikimate and phenylalanine were incorporated (1.0% and 4.3%). However, [1-^{14}C]tyrosine was not incorporated at all (0.02%), and analysis of the enrichment and coupling pattern of **1** (60 mg) derived from [1,2-$^{13}C_2$]acetate (300 mg, 90% ^{13}C, British Oxygen Co., LTD, London, UK) showed that the acetoxy group is derived intact and with high efficiency from a molecule of acetate (Table I). This was clearly not in accord with the original assumption. To obtain further information on the source of the remaining seven carbon atoms of the dihydrofuran moiety, we made use of the efficient incorporation of glycerol (13–15%) and analyzed the labeling pattern of **1** (65 mg) derived from [U-$^{13}C_3$]glycerol[10,11] (300 mg, 99% ^{13}C).

The broad-band ^1H-decoupled ^{13}C NMR spectrum indicated extensive enrichment and coupling throughout the molecule (Table I). The absolute ^{13}C enrichment of the methyl carbon of the 2′-acetoxy group was determined by integration of the methyl proton signal and its ^{13}C satellites in the ^1H NMR spectrum; the integral of the corresponding ^{13}C signal was then used as the reference against which other enrichments were calculated. As expected for the known metabolism of glycerol, pairs of carbon atoms were derived intact from glycerol; these were the same ones which originated from intact acetate units, i.e., the acetoxy group, C-1/C-5 and C-3/C-4. One additional pair of coupled carbon atoms was observed at C-2′/C-3′. A coupled assembly of three carbon atoms, indicative of intact incorporation of a glycerol unit, was detected at C-3″/C-4′/C-5′, as evidenced by a 11-Hz coupling between C-3″ and C-5′. The 4′ signal showed a pattern of at least eight lines instead of the expected four for a doubly coupled carbon. This implied the presence of another coupled two-carbon or three-carbon system involving C-3′, exhibiting the same $J_{2'3'}$. A 2-D INADEQUATE experiment[12] clearly showed that C-3′ was indeed coupled to both C-2′ and C-4′ with a coincident coupling constant of 41 Hz. The projection from the INADEQUATE spectrum at δ 114.68 (C-4′) showed an eight-line pattern, expected for a superimposition of two arrays of three coupled carbon atoms. The two possible arrangements of an intact three-carbon unit involving C-3′ and C-4′, i.e., C-3′/C-4′/C-5′ and C-3′/C-4′/C-3″, were distinguished by spectral simulation (Bruker PANIC routine) using the known δ and J values and proper weighting with respect to intensity. There was close correspondence of the observed pattern to that calculated for the superimposition of a C-3″/C-4′/C-5′ and a C-3′/C-4′/C-5′ coupling pattern and a poor match with the other alternative, C-3″/C-4′/C-5′ plus C-3″/C-4′/C-3′. Another coupled three-carbon assembly was detected at C-1″/C-2″/C-3″. Thus, [U-$^{13}C_3$]glycerol gives rise to two species of **1**, one showing the labeling pattern **a** and the other pattern **b** (Scheme I).

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(1) Konda, Y.; Onda, K.; Hinotozawa, K.; Omura, S. *J. Antibiot.* **1981**, *34*, 9, 1222.
(2) Omura, S.; Kitao, C.; Tanaka, H.; Oiwa, R.; Takahashi, Y.; Nakagawa, A.; Shimada, M.; Iwai, Y. *J. Antibiot.* **1976**, *29*, 876.
(3) Schröder, K.; Zeeck, A. *Tetrahedron Lett.* **1973**, 4995.
(4) Welzel, P.; Witteler, F. J.; Müller, D.; Riemer, W. *Angew. Chem.* **1981**, *93*, 130.
(5) Nakano, H.; Yoshia, M.; Shirahata, K.; Ishii, S.; Arai, Y.; Morimota, M.; Tomita, F. *J. Antibiot.* **1982**, *35*, 760.
(6) Omura, S.; Inamura, N.; Hinotozawa, K.; Otogura, K.; Lukacs, G.; Faghih, R.; Tolmann, R.; Arison, R. H.; Smith, J. L. *J. Antibiot.* **1983**, *36*, 1783.
(7) Werner, G.; Hagenmaier, H.; Drautz, H.; Baumgartner, A.; Zähner, H. *J. Antibiot.* **1984**, *37*, 110.
(8) Goetz, M. A.; McCormick, P. A.; Monaghan, R. L.; Ostlind, D. A.; Hensens, O. D.; Liesch, J. M.; Albers-Schönberg, G. *J. Antibiot.* **1985**, *38*, 161.
(9) Nakagawa, A.; Wu, T. S.; Keller, P. J.; Lee, J. P.; Omura, S.; Floss, H. G. *J. Chem. Soc., Chem. Commun.* **1985**, 519.
(10) Ott, D. G. "Synthesis with Stable Isotopes of Carbon, Nitrogen, and Oxygen"; John Wiley & Sons: New York, 1981; pp 33–35, 37.
(11) Murray, A. W.; Williams, D. L. "Organic Synthesis with Isotopes"; Interscience, New York, 1958, pp 931–932.
(12) Bax, A.; Freeman, R.; Frenkiel, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 2002.

0002-7863/86/1508-0331$01.50/0